

Consumption of Residue Containing Cucurbitacin Feeding Stimulant and Reduced Rates of Carbaryl Insecticide by Western Corn Rootworm (Coleoptera: Chrysomelidae)

ROBERT W. BEHLE

Crop Bioprotection Research Unit, USDA-ARS, National Center for Agricultural Utilization Research,
1815 N. University Street, Peoria, IL 61604-3902

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ABSTRACT Application of insecticide at a reduced rate with a cucurbitacin-based feeding stimulant is a viable alternative to a broadcast insecticide application for control of adult western corn rootworms, *Diabrotica virgifera virgifera*, LeConte. Because of the small amount of material applied, it is conceivable that a high density of beetles could consume all of the spray residue before economic control is achieved. A laboratory experiment was conducted to determine the amount of cucurbitacin-based spray residue consumed by beetles. Dried residue of four treatments were exposed to three groups of 10 rootworm beetles for 1 h each. Treatments consisted of a cucurbitacin-based adjuvant (Cidetrak CRW, Trécé, Salinas, CA) with carbaryl insecticide (Sevin XLR Plus, Rhone Poulenc, Research Triangle Park, NC) mixed at 0, 0.12, 1.2, and 12 g (AI)/liter. For the treatment with cucurbitacin adjuvant only (no insecticide), beetles consumed 0.029 mg beetle⁻¹ h⁻¹ of exposure. Approximately 54% of the beetles were recorded as feeding at any given time during the 60-min feeding period. However, when the spray residue contained carbaryl, no weight loss of treatment residue was measured, though the beetles were observed to feed from the residue during the first few minutes of exposure. When residue included insecticide, beetles quickly ceased feeding (within 20 min), and toxicity behavior was observed 30 min after initial exposure for up to 75% of the beetles, which were classified as moribund (unable to stand upright). Beetle mortality was recorded 24 h after exposure and demonstrated that male beetles (53% dead for three insecticide treatments) were more susceptible to carbaryl toxicity than female beetles (28% dead for three insecticide treatments). Regression analysis showed a significant positive relationship between mortality of female beetles and ovarian development. Based on the measurements of this experiment, it is unlikely that realistic beetle densities would consume enough spray residue to prevent economic control of the beetle population.

KEY WORDS *Diabrotica virgifera virgifera*, cucurbitacin, carbaryl, consumption, mortality

THE WESTERN CORN rootworm, *Diabrotica virgifera virgifera*, LeConte, remains a major insect pest of field corn throughout the mid-western corn-growing states of the United States. The importance of this pest has been verified by the implementation of the Areawide Program for Corn Rootworm Management sponsored by the USDA. The Areawide Program has evolved from integrated pest management (IPM) strategies including intensive scouting and application of reduced rates of insecticide to maintain pest populations below levels that cause economic loss. A key factor in this pest control strategy is the commercial availability of effective reduced-rate insecticide treatments that target the rootworm adults. Controlling the adults before they deposit eggs in the soil precludes the need for a soil insecticide to prevent damage in the subsequent corn crop. At the onset of the Areawide Program in 1996, two products (Slam by Microflow a subsidiary of BASF, Research Triangle Park, NC; and Compel by Ecogen, Langhorne, PA) were commercially available, both which contained cucurbitacin to target rootworm beetles. Cucurbitacin induces root-

worm beetles to feed compulsively on the spray residue (Chambliss and Jones 1966, Metcalf et al. 1980, Ferguson et al. 1983, Metcalf et al. 1987). As a result, rootworm adults can be controlled with extremely low rates of insecticide (Metcalf et al. 1980, Metcalf et al. 1987, Weissling and Meinke 1991, Lance and Sutter 1992) or less conventional agents such as photo-active dyes (Schroder et al. 1998). If the Areawide Program is successful, then corn rootworms can be effectively managed with the application of less insecticide than is required by the current application of soil insecticides.

Unfortunately, both previously mentioned commercial products that contained cucurbitacin to target rootworm adults had problems with application and/or control. Compel was an adjuvant consisting of a cucurbitacin source and a sticker that were mixed with a commercial insecticide on site for application. Special equipment was required for the application of Compel, which made marketing difficult, and Compel was soon removed from the market. Slam remained as the only registered product for rootworm control and

contained both a cucurbitacin source and an insecticide (carbaryl). Promoting area-wide control of an insect with a single insecticide chemistry begs concerns about development of insecticide resistance.

Efforts were begun to develop new spray adjuvants containing cucurbitacins that would impart selective control of western corn rootworm beetles when combined with reduced rates of commercially available insecticides. Two commercial products based on different sources of cucurbitacin became available for application in the 2000 field season. Invite (Florida Food Products, Eustis, FL) contains bitter Hawkesbury watermelon (*Citrullus vulgaris* Schrad.) juice, and Cidetrak (Trécé, Salinas, CA) contains buffalo gourd (*Cucurbita foetidissima* HBK) root powder. Both adjuvants recommend application with as little as 1/10th the lowest recommended rate of commercial insecticides.

In theory, application of feeding stimulant with a reduced amount of insecticide could fail to control high insect populations if the spray residue is totally consumed before all the insects are exposed. Current recommendations for aerial application include applying 111 g active agent, and 222 g Cidetrak (or ≈ 889 g Invite) in 9.35 liter total spray per hectare. In a corn field with an estimated 74,100 plants per hectare and 10 beetles per plant, each beetle need only eat 0.45 mg to completely consume all the spray residue of the Cidetrak application (or 1.35 mg for an application of Invite). Not all spray is deposited where it leaves residue accessible for beetle feeding. With estimates of up to 90% loss of spray deposits, then total consumption of spray residue may become a real consideration for reduced-rate applications, depending on the toxicity of the active agent.

The following laboratory study was conducted to measure residue consumption and subsequent mortality of adult corn rootworms that were fed low-rate insecticide applications containing a cucurbitacin-based feeding stimulant. Cidetrak was selected as the feeding stimulant for this research because it has the lowest recommended rate of application. The goal was to estimate the limits of the reduced rate insecticide treatments with a feeding stimulant to control high populations of rootworm beetles. In addition, we observed differential affects on mortality of male and female beetles when exposed to insecticide residues and subsequently examined ovary development.

Materials and Methods

This experiment was conducted at the USDA-ARS-National Center for Agricultural Utilization Research, Peoria, IL. Beetles for this experiment were supplied by the USDA-ARS National Grain and Insect Research Laboratory, Brookings, SD. Beetles were randomly selected from a mixed-sex colony and were less than 2 wk postemergence of adults when used in this experiment.

Insecticide treatments consisted of reduced rates of carbaryl insecticide (Sevin XLR Plus, Rhone Poulenc, Research Triangle Park, NC) mixed at 0, 0.12, 1.2, and

12 g (AI)/liter. Treatments also included the recommended field rate of Cidetrak CRW at 24 g/liter. The highest rate of carbaryl was equal to 1/10th of the recommended field rate for control of rootworm beetles and represented the current recommendation for field applications with Cidetrak. Previous research showed this rate typically provided over 90% beetle mortality after 24 h continuous exposure (R.W.B., unpublished data) in bioassays similar to the assay described below.

Each of the four treatments containing Cidetrak were applied individually to five preweighed (Ohaus Analytical Plus balance, Ohaus, Florham Park, NJ) glass slides (25 by 75 mm) and allowed to air-dry. Five additional slides were included as a no-treatment control. The application rate of 126 μ l/slide was selected to represent a single-plant application rate, assuming the recommended field application rate of 9.35 liter/ha to a field with 74,100 plants/ha. This volume provided a calculated 0, 0.015, 0.15, and 1.5 mg carbaryl per slide for respective treatment. After air-drying over night, each slide was weighed to determine the amount of residue on each slide before exposure to beetle feeding.

After the treated slides were prepared, a filter paper (Whitman #1, 90 mm diameter) was inserted into each of 75 petri dishes (100 by 15 mm) to be used as test arenas. Beetles were refrigerated for 15 min to facilitate handling before transfer of 10 randomly selected beetles into each of the arenas. The 25 slides were placed individually into petri dishes for 60 min. Each slide was exposed consecutively to three arenas for 30 beetles/slide or 30 beetle h of total exposure. Beetles in these petri dish arenas tend to stay at the circular edges of the dish and typically were not in the center of the areas where the slides were placed. Therefore, the numbers of beetles on each slide were totaled for the five slides of each treatment at 2 min and every 10 min after initial exposure as a measure of the relative ability of the treatment residues to induce and sustain feeding. Also, the number of standing, moribund (alive but unable to stand upright), and dead insects were counted at 10-min intervals during exposure as a preliminary indication of insecticidal activity. After the three exposures, slides were carefully cleaned to remove fecal material from the glass (but not from the area with treatment residue) before reweighing to determine the amount of residue removed by beetle feeding. About 24 h after exposure, the numbers of live, moribund, and dead beetles were counted. Also, the sex of each beetle was determined to indicate differences in beetle mortality. Ovary development was determined for all of the females using a 1–5 scale (Sherwood and Levine 1993).

Statistical analyses were performed using SAS System for Windows, Release Eight SAS Institute 1999). For treatment mortality data, percentages were square root transformed (Steel and Torrie 1980) to verify homogeneity of variance (MEANS < treatment > /HOVTEST = Levene). Because control treatments typically had no mortality and thus no variance, these data were omitted from subsequent analysis of vari-

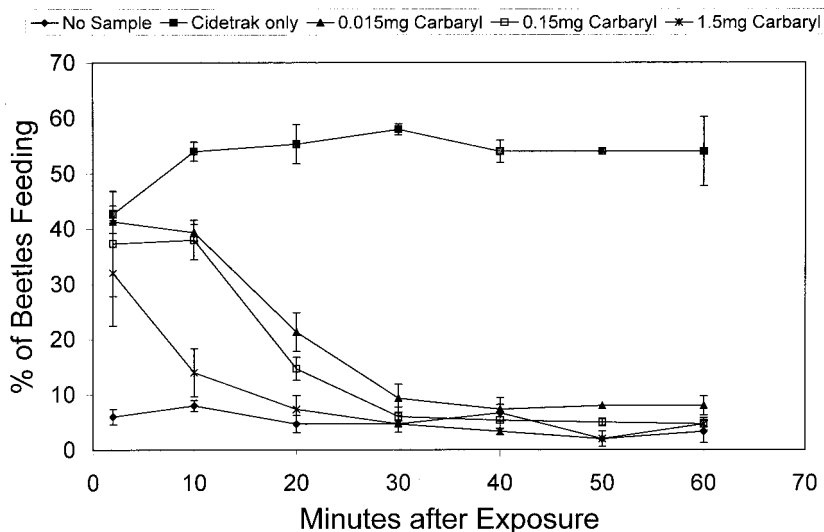


Fig. 1. Percentage of western corn rootworm beetles observed on the slides (feeding) treated with Cidetrak and reduced rates of carbaryl during the 60-min exposure period.

ance (ANOVA) using PROC GLM. Treatment means were then separated by least significant difference (LSD) using the means option. Treatment means were also tested for significant differences from zero using PROC MEANS to verify significant mortality compared with the omitted control treatment. Percentage data for the number of beetles feeding on treatment residue were also square-root transformed and analyzed identically as with the mortality data except that the control treatment was not omitted. Raw mortality data were subjected to linear regression analysis using PROC REG with the stepwise option to include only those variables that significantly affect beetle mortality. Raw data are presented in the tables and figures.

Results

Consumption of Treatment Residue. Despite careful handling, cleaning and weighing of slides, weight loss due to beetle feeding was difficult to measure for treatments that contained insecticide. The slides treated with Cidetrak alone (no insecticide) averaged a weight loss of 0.88 mg after the three exposures (30 beetle h). Based on this treatment, beetle consumption was estimated at $0.029 \text{ mg beetle}^{-1} \text{ h}^{-1}$. Slides with insecticide residue regularly gained weight, ranging from 0.16 to 0.28 mg among the three treatments. This weight gain was the result of insecticide toxicity that caused beetles to regurgitate and deposit feces on the treatment residue, where it could not be cleaned without removing sample.

Feeding Time. Beetles began feeding on the treated areas of the slides soon after exposure. For the Cidetrak-only treatment, 54% of the beetles continuously fed on the residue for the 60-min duration of exposure (Fig. 1). It is not known if the same beetles were

observed on the slides or if there was movement between feeding and nonfeeding for individual beetles. For slides with no treatment, <10% of the beetles were observed on the slides at any given time during exposure. For the three treatments that contained both Cidetrak and carbaryl, the beetles began feeding on the residue similar to those feeding on residues of Cidetrak only. At 2 min after exposure, the no-treatment control had significantly fewer beetles ($F = 7.09$; $df = 4, 10$; $P = 0.006$; $LSD = 2.6$ transformed units) than all other treatments, which were not significantly different among each other. Ten minutes after initial exposure, beetles on slides treated with the two lowest rates of carbaryl were significantly ($F = 30.65$; $df = 4, 10$; $P < 0.001$; $LSD = 1.4$ transformed units) fewer than the beetles observed on slides treated with Cidetrak only. Beetles exposed to the highest concentration of carbaryl stopped feeding before those exposed to the lower concentration of carbaryl (Fig. 1). Thirty minutes after exposure, beetles exposed to carbaryl residue had stopped feeding and the percentages of beetles observed on the slides treated with carbaryl were significantly different ($F = 45.73$; $df = 4, 10$; $P < 0.001$; $LSD = 1.4$ transformed units) from the Cidetrak only treatment, but were not different from the no treatment control slides.

Mortality. Within minutes of exposure, rootworm beetles exhibited toxic effects of carbaryl such as cessation of feeding and loss of the ability to stand (Fig. 2). Beetles exposed to the highest rate of insecticide showed these symptoms sooner than those exposed to medium and low rates of insecticide (Table 1). At the end of the 1-h exposure, 73 and 74% of the beetles exposed to the two highest rates (1.5 and 0.15 mg/slide, respectively) of insecticide were classified as moribund, not being able to stand. Significantly ($F = 31.48$; $df = 14, 60$; $P < 0.001$; $LSD = 10.1\%$) fewer

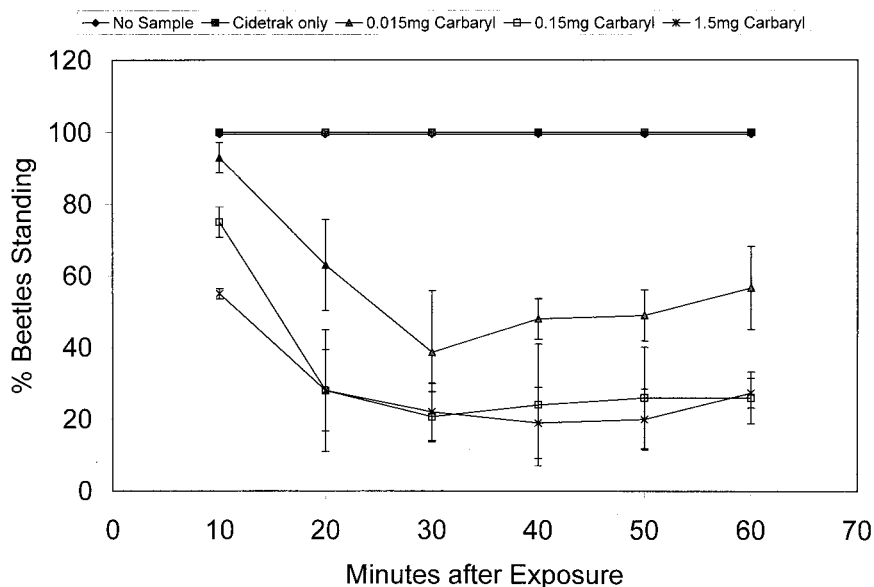


Fig. 2. Percentage of western corn rootworm beetles not visibly affected by carbaryl toxicity (standing) during the 60-min exposure period to treatment residues of Cidetrak adjuvant with reduced rates of carbaryl insecticide.

beetles (43%) exposed to the lowest rate (0.015 mg/slide) of insecticide were classified as moribund at this time. However, 24 h after exposure, only 56% of the beetles exposed to the highest insecticide concentration were classified as dead or moribund, indicating some recovery by the beetles after exposure had ended. Beetle mortality 24 h after exposure significantly ($F = 17.92$; $df = 14, 60$; $P < 0.001$; $LSD = 8.0\%$) increased for groups of beetles exposed to higher rates of insecticide (Table 1). The percentage of dead beetles for the Cidetrak only treatment was not different from zero ($t = 1.47$, $P = 0.164$) indicating that Cidetrak does not have insecticidal activity.

Sex Ratio. The sex ratio of the beetles used in this experiment favored females (61%) (Table 2). In general, females were less affected by the insecticide than the males. Of the female beetles exposed to insecticide, only 28% were classified as dead or moribund after 24 h. In contrast, 53% of the male beetles exposed to insecticide were classified as dead or moribund. Thus, male beetles appeared more susceptible to toxicity than female beetles. Three beetles escaped the petri dishes during transfer of the slides and two were

eaten by remaining live beetles before their sex was determined and thus were not counted. Estimations of ovary development of the females showed that there was no significant difference in the average development among treatments ($F = 1.84$; $df = 14, 30$; $P = 0.18$) (Table 2). No differences were expected because insects were randomly selected from a single cage of beetles. However, regression analysis indicated significant positive relationships between beetle mortality and both the rate of insecticide (slope estimate = 313% mortality/g, Partial $R^2 = 0.448$, $P = 0.001$) and ovarian development stage (slope estimate = 12% mortality/stage, Partial $R^2 = 0.116$, $P = 0.017$). This regression analysis suggests that female beetles with more developed ovaries were more sensitive to carbaryl toxicity given the exposure conditions of this experiment.

Discussion

Results of this study provide information concerning the potential for total consumption of reduced-rate insecticide applications with cucurbitacin-based

Table 1. Percentage of *D. virgifera virgifera* beetles not exhibiting toxic effects (standing) of carbaryl insecticide applied with Cidetrak CRW feeding stimulant and percentage mortality 24 h after exposure

Carbaryl rate, mg(AI)/slide	% standing, 10 min	% standing, 1 h	% standing, 24 h	% mortality, 24 h
(None)	100a	100.0a	99.3a	0.7d
(Cidetrak only)	100a	100.0a	98.0a	0.7d
0.015	93a	56.7b	84.2b	11c
0.15	75b	26.0c	59.3c	23b
1.5	55c	25.3c	43.9d	49a

Means (based on 150 beetles exposed for each treatment) in a column followed by the same letter were not significantly different, $P = 0.05$, LSD .

Table 2. Numbers of male and female *D. virgifera virgifera* adults with and without visual symptoms of carbaryl toxicity 24 h after exposure in the feeding experiment and average ovary development based on a 1–5 scale

Carbaryl/rate, mg (AI)/slide	Without symptoms		With symptoms		Total no. beetles	Avg. ovary development ^a
	♂ ♂	♀ ♀	♂ ♂	♀ ♀		
(None)	54	94	1	0	149	ND
(Cidetrak only)	53	92	1	1	147	ND
0.015	43	82	14	11	150	2.00
0.15	27	62	37	24	150	2.32
1.5	14	51	45	39	149	2.37
Total	191	381	98	75	745	ND

^a ND, not determined.

feeding stimulant. Each treated slide was exposed to 30 beetles for 1 h each, 30 beetle h of exposure. We estimated that 54% of the beetles were feeding during the exposure period for an estimate of 16.2 beetle h of feeding, based on the Cidetrak treatment with no insecticide. During this exposure, 0.88 mg weight was removed for an estimated consumption of 0.029 mg beetle⁻¹h⁻¹ of exposure or 0.054 mg beetle⁻¹h⁻¹ of feeding. At this rate of feeding, the hypothetical density of 10 beetles per plant could consume the spray deposits in less than 10 h. Numerous additional calculations can be made based on varied assumptions of application rates and beetle densities. The no-insecticide treatment represents a worst case scenario similar to a slow-acting insecticide, which would allow beetles to feed for an extended period of time before affecting the insect.

One striking observation of this experiment was that the beetles exposed to insecticide treatments quickly stopped feeding (Fig. 1). Reduced feeding time suggests that much less of the spray residue would be consumed when compared with the Cidetrak only treatment mentioned above. To determine the amount of material to apply to the slides, we estimated the amount of spray per plant given recommended field application rate of 9.34 liter/ha. In our assay, this treatment residue for the recommended field treatment (112 g/ha) with Cidetrak killed 15 beetles with no detectable loss (visual observation or weight) of residue from the slides. Thus, no specific calculations can be made from these data even though the obvious conclusion supports the contention that recommended application rates are sufficient to control beetle populations. In a similar preliminary experiment with carbaryl rates 3 times higher than used in this experiment, 50 beetles (of 60 exposed for 30 min each) were killed with ≈0.2 mg weight loss of treatment residue (R.W.B., unpublished data). Based on these values (50 beetles killed for 0.2 mg residue consumed), a single plant application of 126 μl (9.5 mg residue), could control 2,375 beetles. Even with 90% loss of spray residue, this application could conceivably control >200 beetles per plant, a density about 285 times greater than the 0.7 beetles per plant treatment threshold (Tollefson 1997). As a result, it is likely that low-rate applications of baited insecticide will provide sufficient material to control high densities of beetles under field conditions.

Beetle recovery after exposure to residues of reduced carbaryl rates indicates that there are limits to rate reductions. Rapid cessation of feeding and initial observations for mortality (1 h after the initial exposure) indicated that even the lowest rates of insecticide affected the exposed beetles. Although ≈43% of the exposed beetles were classified as moribund at the end of the 60-min exposure to the lowest carbaryl rate, many recovered leaving only 11% dead plus 5% moribund 24 h after exposure. This observation indicates that there is a minimal concentration of carbaryl in the residue required for effective control of the beetles, and field applications should not use less than the label recommendations for insecticide rate reductions. The effects of sublethal exposure to carbaryl on longevity and reproductive capacity of recovered beetles were not measured in this experiment.

Observations from this experiment raise some issues that require additional study. Beetle recovery from subtoxic exposure combined with cessation of feeding could accelerate the development of insecticide resistance. Selection for insects, which do not feed sufficiently to ingest toxic levels of insecticide, suggests the possibility for the development of modified behavior mechanisms for resistance. However, this feeding stimulant is a spray adjuvant and can be used with a variety of commercial insecticides allowing for rotation of active ingredients. By rotating active agents with alternative modes of action, the development of resistance and cross-resistance should be avoided or delayed (Brattsten et al. 1986). Using a feeding stimulant adjuvant for resistance management was not the focus of this experiment. Yet, the data presented here suggest a need for additional studies on this topic.

Our supporting observations on sex ratios and ovary development provided results to direct future basic research in insect toxicology. Higher percentage mortality for male beetles compared with female beetles may have been the result of relative dose exposures. Male corn rootworm beetles are smaller than the females and thus the amount of carbaryl consumed per weight may account for the differences in mortality. Likewise, the relationship between mortality and ovary development poses interesting questions, which might relate changes in metabolism with different levels of toxicity due to carbaryl. Additional knowledge related to these topics may be useful in explain-

ing unexpected results in field populations treated with reduced rate insecticides and feeding stimulants.

In conclusion, this experiment demonstrated that applications of reduced rates of effective insecticides with a feeding stimulant is likely to provide enough spray residue to control high beetle populations. However, beetles feeding on the no-insecticide Cidetrak treatment consumed $0.054 \text{ mg beetle}^{-1} \text{ h}^{-1}$ of feeding, a rate at which realistic field densities could consume all spray residue in less than a day if there is no limiting affect provided by the insecticide. It was also observed that all reduced rates of carbaryl (down to 0.12 g/liter) initially caused beetles to stop feeding on spray residue containing Cidetrak. Beetle mortality was significantly lower as the rate of carbaryl was reduced, suggesting that efficacy of control will be reduced if applicators do not meet the minimal insecticide rate recommendations of the Cidetrak label.

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